

# Etre ou ne pas être un microRNA

## *L'existence précaire d'un régulateur capital*

Daniel Gautheret

Que faut-il pour qu'un microRNA fonctionnel s'exprime? Peu de choses en fait. Les enzymes qui forment les miRNA reconnaissent des structures tige-boucle très fréquentes dans tous les génomes et qui se retrouvent dans de nombreux transcrits. Je montrerai avec quelle facilité les microRNA apparaissent et disparaissent au cours de l'évolution, puis j'expliquerai comment on peut exploiter les méthodes de comparaison de structure pour tenter de mieux cerner l'essence d'un microRNA. Je présenterai enfin notre serveur d'évaluation des candidats microRNA "miReval".

# What is a microRNA ?

- Any transcript
  - Pol II : Independent, intron, UTR, viral
  - Pol III (from *Alu* elements)
- Rnase III (Pri-miRNA Drosha / pre-miRNA Dicer)
  - Rnase IIIs will cleave anything suitable
  - See for instance pseudogene/gene hybrids (2 papers in Nature 2008)

# Easy made, easy lost

- Numerous non-conserved, « species specific » miRNAs
  - (cf apes studies)
  - implies a fast rate of apparition / loss



# What does Dicer need?

- Unpaired positions at base of stem
- Basepair sequence unimportant
- Lai et al. 2003: minimal pre-miRNA stem is 23bp long

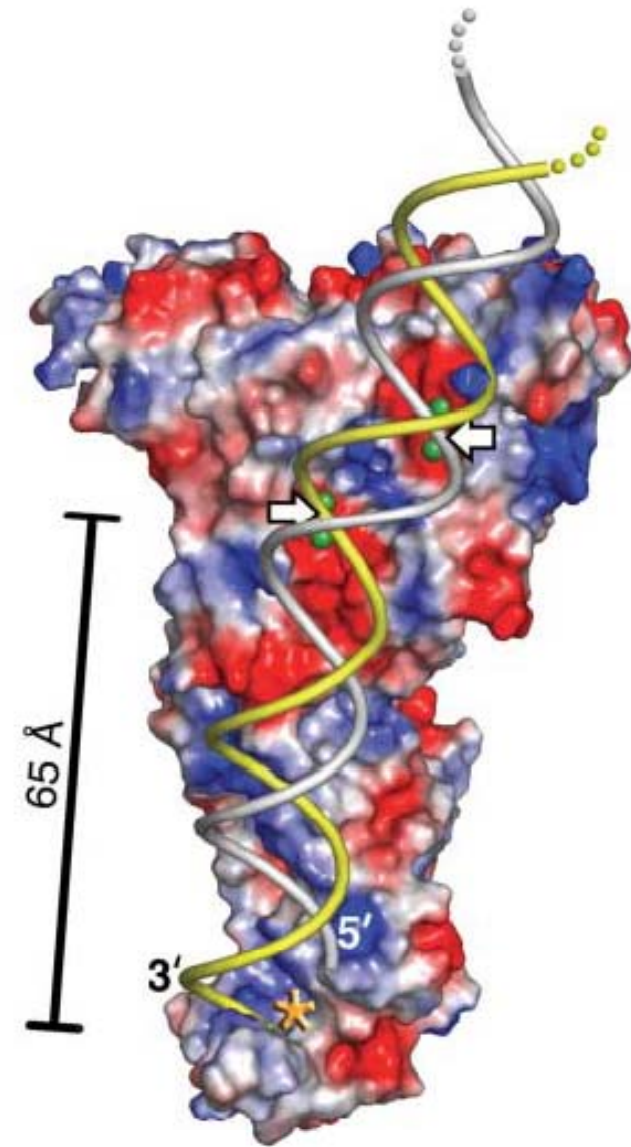
**TABLE 1.** Selected miR-30 mutants

	miRNA precursor	miRNA expression
miR-30	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-CGU GACGUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	+
miR-30(ΔAGAUG)	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUG A 3'-CGU GACGUUUGUAGG—CUGACUUUCGG CAC G </pre>	±
miR-30(UGAGA)	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUGAGA 3'-CGU GACGUUUGUAGG—CUGACUUUCGG C </pre>	+
miR-30(GCG)	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-CGU GACGUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	+
miR-30(ACAGCG)	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-CGU GACGUUUGUAGG—CUGACUUUCGG ACAG </pre>	+
miR-30(ΔUC)	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-CGU GACGUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	+
miR-30(G)	<pre> 5'-GCGGCUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-CGUCGACGUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	+
miR-30(UC)	<pre> 5'-GCG CUGUAAACA C GACUGGAAGCU GUGAAG 3'-CGU GACGUUUGU G—CUGACUUUCGG GUAGAC </pre>	±
miR-30(GA/UC)	<pre> 5'-GCG CUGUAAACAGAC GACUGGAAGCU GUGAAG 3'-CGU GACGUUUGUCUG—CUGACUUUCGG GUAGAC </pre>	+
miR-30(A)	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-CGU GACAUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	+
miR-30(C)	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-CGU GACGUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	+
miR-30(GAG)	<pre> 5'-GCG CUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-GAG CACGUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	-
miR-30(CUC/GAG)	<pre> 5'-CUC CUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-GAG CACGUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	+
miR-30(CG)	<pre> 5'-GCG GUGUAAACAUC C GACUGGAAGCU GUGAAG 3'-CGU CACUUUGUAGG—CUGACUUUCGG GUAGAC </pre>	+

Zeng & Cullen 2003. Mutagenesis of pre-mir-30

## In 3D...

- 65Å between PAS domain that recognize 3' end and Rnase III domain.
- ie. 25 nt
- miRbase has 15bp precursors



MacRae et al. 2004. Crystal structure of Dicer with model dsRNA

# Human genome: one 33bp stem every 10kb!

If 90% of the genome is transcribed (Encode):  
150-300.000 potential miRNAs. Wow!

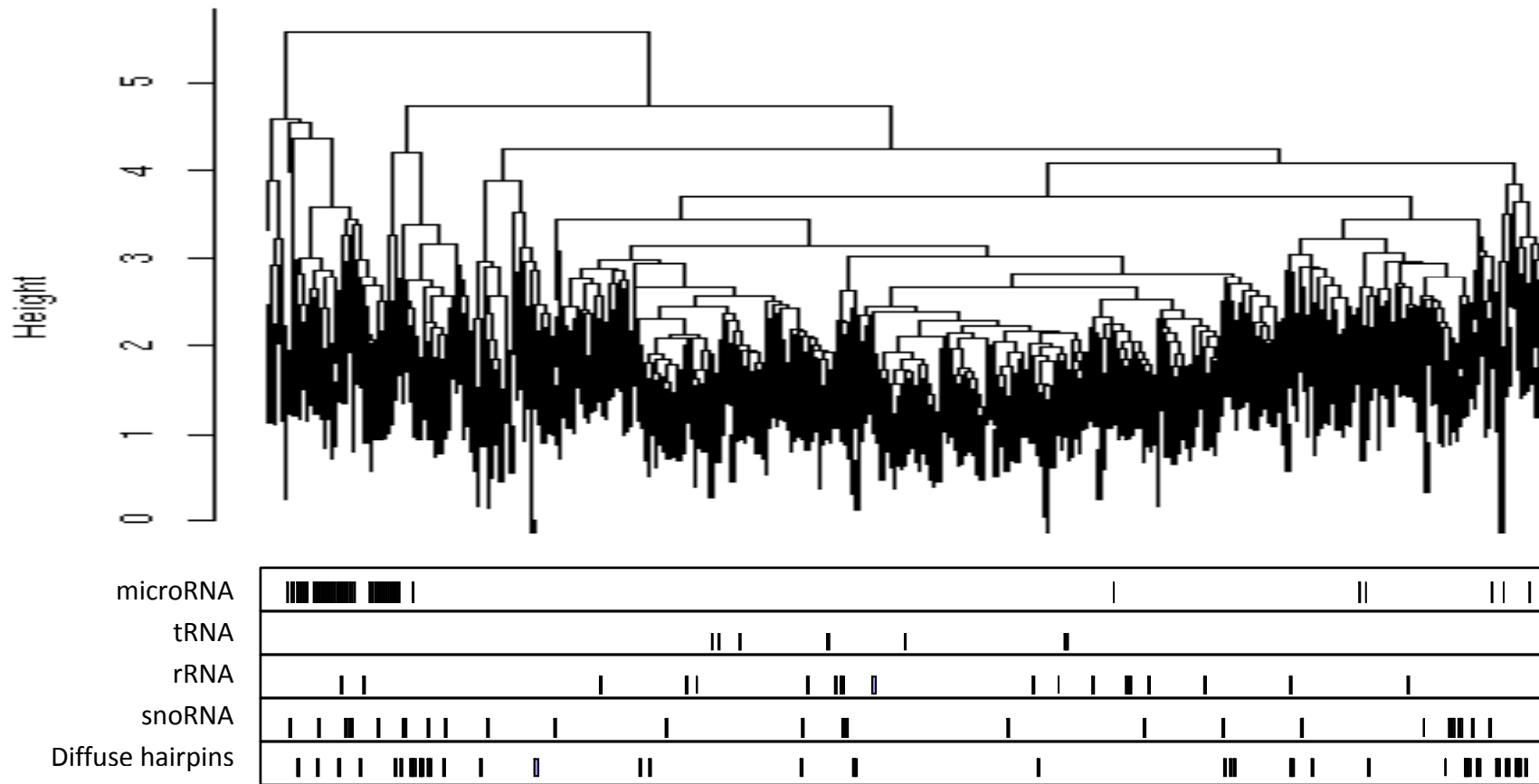
```
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 60001 128
. gtgcaagg atacagggtcctctgtcccaagctctg agcctgtcctgccatcccagggtgccccatctgcactctccagacttcatcccctgcaagg tggggctggggatgggctctgct gg cectgccc
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 111735 116
. toatggtagtgcctgct gctggccccagggtt agggctgctcagcagctcctggctggaaaggatgaatgtcccacaagc agctttggggcctgc tt ggtgggagcagccggagg
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 113267 156
. ttgtgctttgttttgcct tttttagctacatgg tttatgtgaggtggggacttaacttgagttgctctgtctttacaggagacttgcctttttctctgctggttgttttcaacttttgcctct ctgtttagaagaaa c attagaactggggtgtgg
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 144296 98
. catctc ttccaggggccagggtgcaacacagg cctcccagctccgtccaacagcagcctctct tttgtctgaggctgggctcttggggag g aaggtg
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 161278 92
. gtgtcc gagacactttcaacagaaagtgaagac ctcaaggggagaagcactctcaag gcttgtgttttttggtagaggtgtctt tg gggatc
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 240005 74
. ttgtgtgttt aatattttattagattggat gggtaa gtccaaagttgaaacagagatt ca aaacaacatga
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 416597 80
. tgcctgca tccagaaacagagagtgattgcat gaacatggctaac atgcagttggttctctgctgctggg g tgcaggca
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 439681 141
. ggtgcaatgocgggtggggta caccctgtttt gaaaaaagtgcacatagatggtgcagatgcaagcctctgattatgggcttcttctctgcccacatgtgtatg aagataaggatg c tattttattggcttatatt
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 30 444584 129
. acctggctgctgatgtccatttggaaactg gact ccaactgggaactggcatacacctaaagacatagttgagccgggtgtttgatgtccaat gtc ac caggctccaggtggacaccgggctcaggt
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 473167 147
. ggaggctggt ggcgggtggggtggggtggggt tggggctccagcttctaatgttctaatgtgcagggcttggcctgaccagctcctaccctggaacctttccaggcccat gctcctccctggggtggc t gttggagttt
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 30 507654 113
. tatgtgcccacaactccca tgtatgtgtgcctgag tatgaacatggtgtgaaacacacacatacactcctcctcctcct catgagtatgtgtgca cy tgggtggtggggtgctgt
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 574824 82
. attcttttagttttttaatt aaaaaacaaa ctcaactccattctt tttatttttt tt aattgaggtaaacttaagctt
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 759839 152
. gcca gggctgcccctcccggccggcactcccc actcaccatcctacggggcccaaaagctgctcctcctcaacaaggactcccagagaccacggcctcctgctgacctgcaga ggtgggtgcccggagggtgggggtggct cc tggc
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 764966 94
. ggggcaaggggcggggggggcctgg gggctgg gctctccctgcaagctcaactacacag ctggcct g ccaaggccttggagcttcccagctt
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 768893 162
. tgag tgaatgggcaagggtgccccctgctct ctgcatctctgagtgaggtggggctgccccctgctctctgctcctgagtgagtgggggggggctgccccctgctctctgtgctcctgagtt ggggtgggggtgcccctctgctctctgtg ct cttg
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 30 778757 136
. totcttagtattggggcaggtgggcaagae ctgt ctctctctcctgctcactgaggtattggtgatgacatgggggtggctgaggtctgcccctgtgctc gcat at gttctggatattgggctagttgtggggg
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 866505 100
. gtgcaatgctgtgtggcgg tgggtgtgcaaggcg ggggcaaggacacagatgcaacctcaagatc cgtgtgtgcaacca g tgtttgtgcatgtgcaaac
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 31 885595 133
. ttgctc caggtatgtgcaacaggtgtgaacacgct tccaacagggcagtggaactgtgcaacatgctcctcaacaacactgtggcgcaaggcaaggttccc acatgtgttcaatgcttgcgcaacatgcaag t gggctg
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 888619 113
. ttataaaag tggtagagtgattgcaattcaac ataaacagtgagctgtcttctggtatctagcagacaacttaacttca gaaatgcaatcatttttacta g ctttgagaaa
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 916516 120
. gttctgggaacatgtcaac attaggtgtgtg tgggtatgtgtgtattaggcgggggtggggtgggtgtgtgtgtgcaagtc tgcagatttgcct t gtaatgtgtgtctatgtgt
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 978532 145
. gagagctggcaggtagtgagg ggccttgggag ctctcaggtgaaactggggctgtctccaaggtaccaggtgggggtgggctgggctcactcaaacctaaggcc tttctgggtct ag cattattgcttctgcttct
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 974771 71
. tctctgctgcaattct tgggctctgctggcctg tatg tagtctggcgggacacta c atagtgcaagttggg
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 966125 120
. tggttctctgct ccaagatcagctgctcagc aaggaccatgtggaggcccagcactggagcagttctgtaagctgctgcttcc tggaggccaaggttctag ca aggtgggtatcca
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 909721 151
. gggcgctctctgtctgc tcaccccgggggggg gaggacagcagacagccccagcctgctctggcgggagtggtgcttagaccaggtcctgctgggcttggcccagagacc cctgctctgggggag cc gttacgggcaagactcc
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 706711 159
. ggggcccagg ctctctctgtggaagcttcc ctctctgctcctctcttccactgcccagggcctgcaagcctcctgcaagcctcctgcaagcctcctctctcctcaacactctctgccc gtgggcttgcagacggagggtg gc cctggtatcc
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 693711 86
. aggtgagtggtgtactgtccgg ggggtatgt gcatcactctgggggggta gttgtttct g cgggggggtgagtgatgcaactg
. >i|29806588|ref|NT_011519.10|Hs22_11676 Homo sapiens chromosome 22 enomi oni, referene ssembly(1000001->2000000) 29 652929 98
. tttccagctgggttaactgcaaca cgttatgt tcaatcaagtgctcctgctcaggggtgca cgtataga ca tttgtgtgtgctgggcatgtgtag
```

# Question

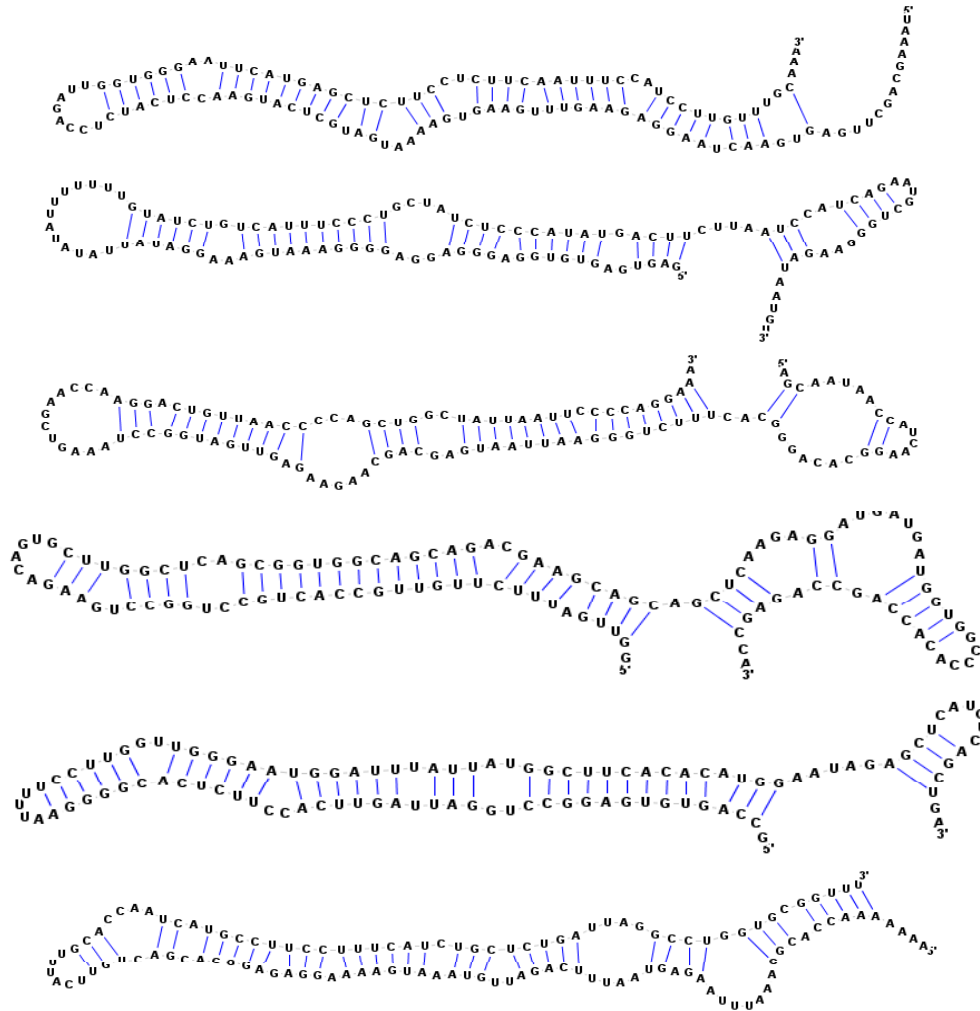
- Can we differentiate true miRNA precursor at the structural level alone?
- We've tried to answer this using the structure comparison tool *Rnaforester* from Robert Giegerich's lab



- Take 1200 human RNA structures from Washietl et al's RNAz screen
- Cluster using RNAforester



# « Diffuse hairpins »

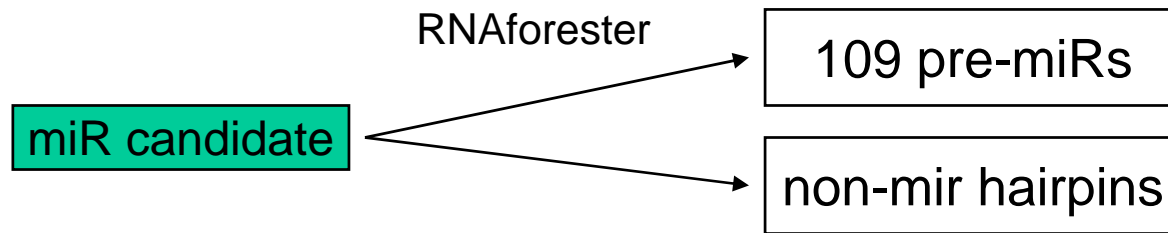


- Same average length as pre-miRNA
- Same average  $\Delta G$
- What's special with them?

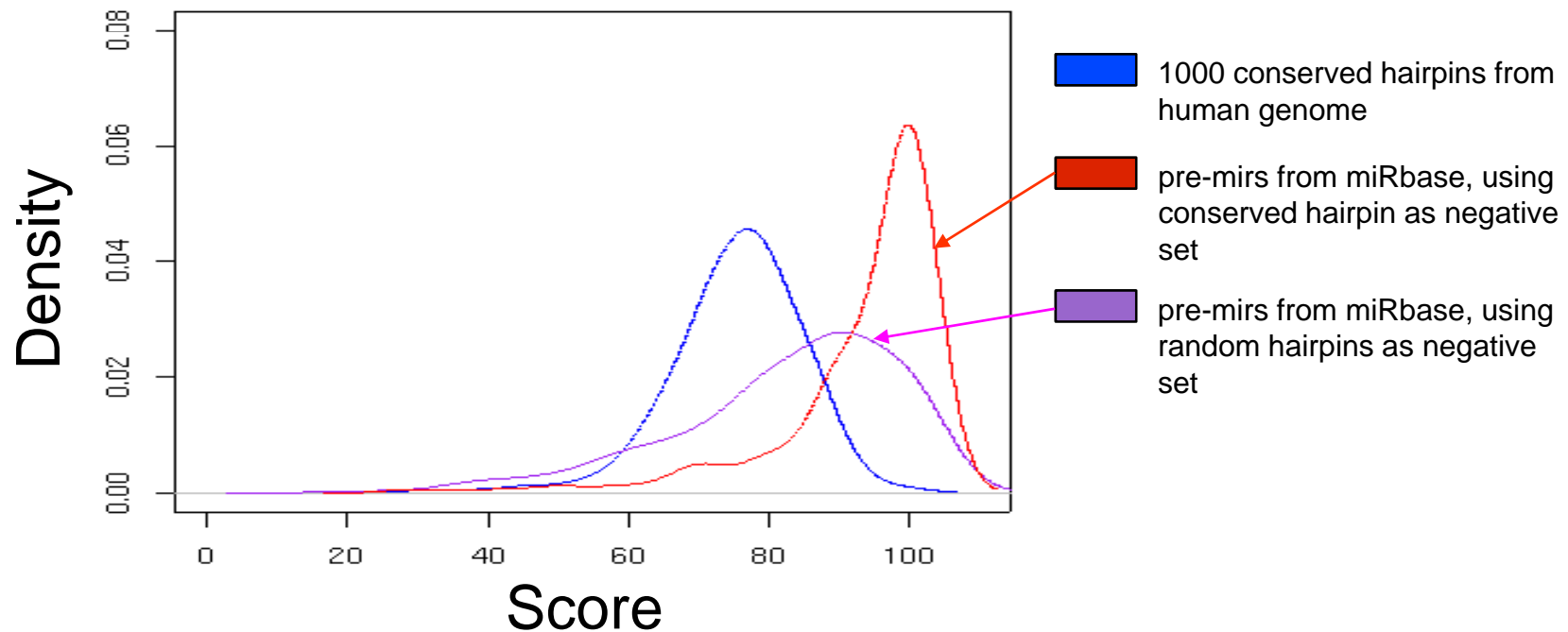
# Compare precursors / non-precursors

**TABLE 1.** Characteristics of miRNA precursor and nonprecursor stem-loops

Parameter	Mean value, precursors	Mean value, nonprecursors	Difference P value
Bulge dist from apical loop (nt)	8.8	9.8	0.42
Internal loop dist from start (nt)	9.43	10.43	0.4
Internal loop dist from apical loop (nt)	8.74	7.80	0.4
Total number of bp	31	29.2	0.21
Bulge dist from start (nt)	16	11.45	0.003
$\Delta G$ (kcal)	-33	-29	0.002
Max consecutive bps in stem	9.38	8.33	0.00015
Number of internal loops	3.12	3.89	1.6e-7
Apical loop size (nt)	7.1	13.1	6.8e-10
Internal loop size (nt)	1.94	3.20	3.8e-10
Number of bulges	1.86	3.29	2.2e-16
Bulge size (nt)	1.47	4.37	2.2e-16



Top 20 hits  
 Score=0 if diffuse  
 Score = 1/rank if miR  
 Sum scores  
 Scale to 100



Conserved/expressed hairpins differ more from a pre-miRNA than random hairpins. Expressed hairpins dodge the miRNA pathway!

# Structure as a miRNA detection criteria

- Set 1: closely related sequences
- Set 2: distant sequences (no primate or no *Caenorhabditis*)

**TABLE 2.** Micro RNA prediction performances of the programs Triplet-SVM, BLAST, and miRAlign (references in text) compared to secondary structure clustering score

Precursors tested	Method <sup>a</sup>	Sensitivity	Specificity
Human	Triplet-SVM (set 1)	0.92	0.76
	Triplet-SVM (set 2)	0.86	0.74
	BLAST (set 1)	0.87	0.99
	BLAST (set 2)	0.63	0.96
	MiRAlign (set 1)	0.92	0.99
	miRAlign (set 2)	0.69	0.99
	<u>Structure clustering</u>	0.80	0.76
<i>C. briggsae</i>	Triplet-SVM (set 1)	0.90	0.75
	Triplet-SVM (set 2)	0.88	0.74
	BLAST (set 1)	0.84	0.98
	BLAST (set 2)	0.06	0.95
	MiRAlign (set 1)	0.90	0.99
	miRAlign (set 2)	0.10	0.99
	<u>Structure clustering</u>	0.79	0.75

# Conclusions

- Compared to other genomic hairpins, miRNA precursors have
  - Shorter internal loops, shorter bulges
  - Fewer bulges
- Raw structure is an important part of ncRNA identity
- Expressed sequences tend not to resemble miRNA precursors
  - Better for them
  - And for us !

# Homology-search for new miRNAs

- Blast?
- Other tools?

# ERPIN: Profile-based RNA search

## Training set

>H.zapienz SclD  
 >M.murculur SclD  
 >D.melanogaster SclD (SPS?)  
 >H.zapienz SPS2 HGI/TH0758351 (5)  
 >M.murculur SPS2 MGI/TC382385 (5)  
 >D.melanogaster SPS2-like DGI/TC85266 (5)  
 >T.negriviridis SPS2-byzim TetradGenome/COB5119BF05SP1  
 >F.fubripes SPS2-byzim FuquGenome/Scaffold\_3206 (9)  
 >C.intestinalis SPS2-byzim ctanaEST/D000440482H02CP1(9)  
 >T.muriz SPS2-like EMBL:BM217798 (9)  
 >D.melanogaster SclG  
 >D.melanogaster SclG

```

TTCTG -TTA--A TGAAGTCTCTCCCT CTAAA -C CCC ATTAA- GGA C----- T GGGAGGGCAGAG ---CAAGCCT----- CAGAA
CTCTG ATA---A TGATGCTCTCTCCCT CTAAA -C TCC CAGTAA GGA C----- T GGGAGGGCAGAG ---CAAGCCT----- CAGAG
TTCAA CTT---A TGAGGATTATTCT -TAAA -G GCC TCT--- GGC T----- GGAATAGCTGAA ---CCTTA----- TTGTA
GTCTC TGT-TAA TGAAGTCTCTCCCT CTAAA -C CCC ATT-AA GGA -C----- T GGGAGGGCAGAG ---CAAGCCT-C-----A GAGCC
CTCTG ATA---A TGATGCTCTCTCCCT CTAAA -C TCC CAGTAA GGA -C----- T GGGAGGGCAGAG ---CAAGCCT----- CAGAG
CATTG AAC-TTA TGAGGATTATTCT -TAAA -G GCC TCT--- GGC -T----- C GGAATAGCTGAA ---CCT----- TATTG
GTCNC CTC-CAA TGATGCTCTCTCTG -CAAA -C TCC CC-G-T CGG GGT----- C GGGAGGGCAGAG ---GC----- GTGCC
CTCC TCT--AA TGATGCTCTCTCTG -CAAA -C CCC AGCCGA CGG GGT----- C GGGAGGGCAGAG ---GTGT-GC--CTTC GGATC
AGCAG CTT--AA TGAGGATTATTCT -TAAA TT TGT ATGAAA ACA --A ---AGG AAGTGGGATTGAA ---GCC----- CTGCT
T GCA CAGCCTG TGAGGATTATTCT -TAAA -G CCT ACAG-- AAG --C----A G CBTGAGTCTGAT ---GCTCTG----- TGACA
GTGCC CGC-TTA TGAAGGATTATTCT -TAAA -C TCC AAGT-- CGA G-C----- G GGAATTGCTGAT ---TACGATTA-- ACCAC
GTGCP GAT-TA TGAAGGATTATTCT -TAAA -C TCC AAGT-- CGA G-C----- G GGAATTGCTGAT ---TACGATTA-- ACCAC
  
```

Helix profile  
(16xN)

A:A			
G:A			
C:A			
U:A			
A:G			
G:G			
C:G			
U:G			
...			
U:U			

$S_{b1,b2} = \log(F_{b1b2} / F_{b1} \times F_{b2})$

Single-strand  
profile (5xN)

A			
G			
C			
U			
-			

Search algorithm combines  
**dynamic programming & profile search** for single strands and  
**profile search** for helices

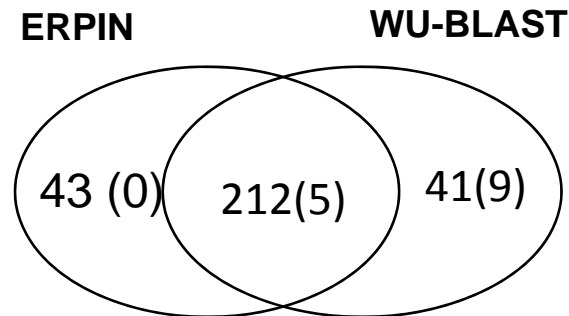


# Dataset for search

- 18 miRNA families from RFAM
  - 10 sequences in average
  - Aligned using CLUSTALW + basepair pred with RNAfold

# ERPIN vs WU-BLAST

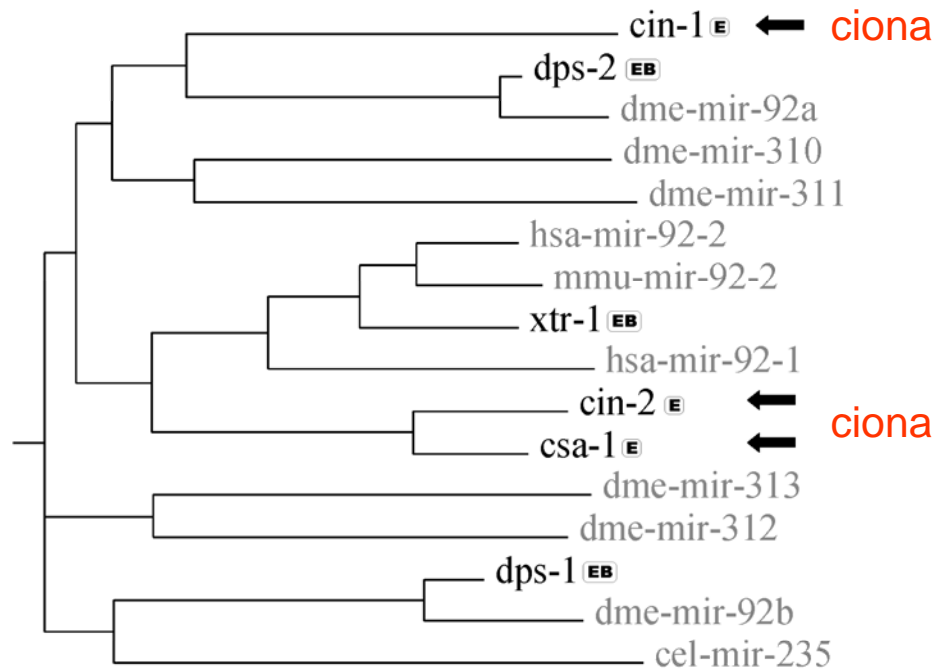
- 20 animal genomes scanned
- Sensitive WU-BLAST parameters (W=7)
- E-value  $\leq 0.01$



miRNA families	sp	Genomic sequence name	Position	E-value	New miRNA precursors sequences
miR-133,	mo	Chromosome 9	+ 13549277-13549341	4.41 10 <sup>-7</sup>	TC t GGCTGGT caaac- GGA a CCAA gtcogtcttctgagaggt--- TTGG TCC CCTTCA ACCAGCT a CA
miR-145	tnt	COAG1445DC08LP1	- 86-151	1.06 10 <sup>-3</sup>	TG t GGCTGGT caaac- GGA a CCAA gtcaggtgtttctgtgaggt-- TTGG TCC CCTTCA ACCAGAC t AT
	tnt	COAG790DH08SP1	- 127-192	2.39 10 <sup>-7</sup>	TG t GGCTGGT aaaac- GGA a CCAA gtcaggtgtttttgtgaggt-- TTGG TCC CCTTCA ACCAGCT a TG
	xtr	scaffold_7052	- 12019-12083	2.64 10 <sup>-6</sup>	TG c GGCTGGT gaaaa- GGA a CCAC atcaaccagaaaaaggat--- TTGG TCC CCTTCA ACCAGOC g CA
	xtr	scaffold_60773	+ 1544-1608	1.90 10 <sup>-7</sup>	TA t GGCTGGT caaac- GGA a CCAA gtcogtcttcttagaggt--- TTGG TCC CCTTCA ACCAGCT a TT
	dre	Contigctg10804.1	- 159936-160001	2.00 10 <sup>-4</sup>	AG t TGCTGGT aaaac- GGA a CCAA gtcgggtgtttgogagaggt-- TTGG TCC CTTTCA ACCAGCT a CT
	dre	ContigBX276101.6	- 114530-114595	4.16 10 <sup>-6</sup>	TG t GGCTGGT caaat- GGA a CCAA gtcaggtgtttctgogaggt-- TTGG TCC CCTTCA ACCAGCT a CT

# New homologues of a miR Cluster

## miR17 cluster



Grey: initial training set

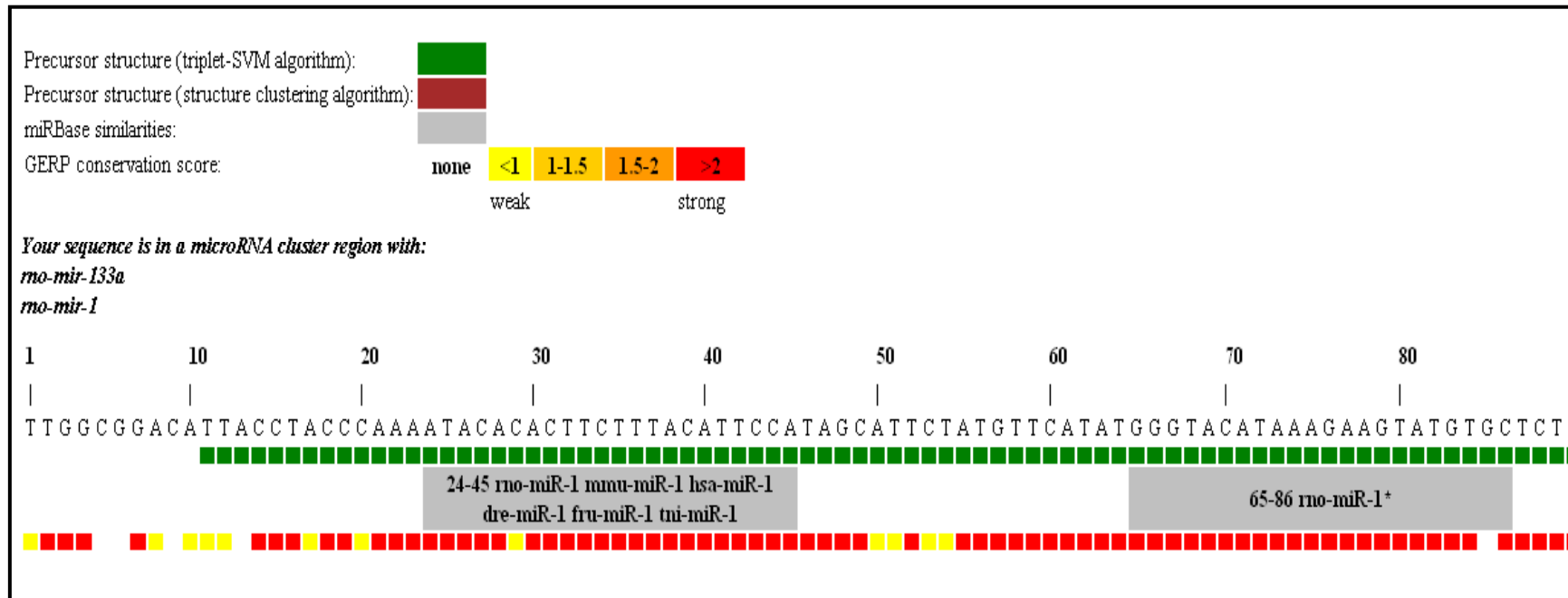
“E” indicates hits identified by ERPIN only,

“EB” indicates hits identified by both ERPIN and BLAST.

# The mirEval webserver

Ritchie & Gautheret, Bioinformatics 2008

- [tagc.univ-mrs.fr/mireval](http://tagc.univ-mrs.fr/mireval)
  - SVM+clustering+homology+conservation



# MirEval Analyzes: 1. Structure

- Triplet SVM (Xue et al. 2005)
  - Positive/negative training set (human)
  - Uses word frequencies statistics to classify sequence into pre-miR/other
- Structure-based classifier
  - Our RNAforester-based clustering

## MirEval Analyzes: 2. Conservation

- Genomic Evolutionary Rate Profiling (GERP)
  - Blast vs Genomes
  - Retrieves Genomic location
  - Get GERP score from Ensembl
  - (Score is based on 10-way Vertebrate alignment)

## MirEval Analyzes: 3. Gene location

- Location in a miR cluster?
  - Retrieve genomic location
  - Retrieve all annotated miRNAs in Ensembl region

# MirEval Analyzes: 4. Homology

- Homologous miRNAs?
  - Slice sequence into 1000 nt segments
  - Blast vs miRbase using online tool



# Reduced maintenance

- All analyzes outsourced, except structure
- 149 000 mirEval runs between dec 2007 and sept 2008.

```
[ritch@obelix mireval]$ wc logs  
149139 2888970 23740472 logs
```

# Merci!

The screenshot shows a Mozilla Firefox browser window displaying the website for the Institut de Génétique et Microbiologie UMR 8621. The browser's address bar shows the URL [http://www.igmors.u-psud.fr/?var\\_mode=recalcul](http://www.igmors.u-psud.fr/?var_mode=recalcul). The website header includes navigation links: [Contacts](#), [Plan d'accès](#), [IFR 115](#), and [Annuaire UPS](#). The main content area is divided into several sections:

- Présentation de l'institut:** A text block describing the institute's location on the Orsay campus, its history (founded in 1989), and its research focus. It mentions 24 teams, 73 researchers, and 30 doctoral students.
- À noter:** A list of recent events and reports, including "Campagne NOEMI d'hiver 2007", "Evaluation biennale des chercheurs", "Compte rendu annuel d'activité des chercheurs", and "Concours chercheurs 2008".
- Actualités:** A section for news, featuring a "Prochain séminaire" on December 4th at 11H30, presented by Stéphanie Marsin from CEA-DSWDRR. The topic is "Identification des voies de recombinaison homologue chez la bactérie *Helicobacter pylori*".
- Thèse à venir:** A section for upcoming theses, with one listed for Monday, December 23, 2007.

The left sidebar contains navigation menus for "La Recherche", "L'Enseignement", "L'Information Scientifique", "Les Services communs", "Annuaire", and "Intranet". There is also a search bar and a language selector set to "français". The browser's status bar at the bottom left shows "Terminé".

IGM, Orsay